Homocysteine and Cerebral Infarction in Finnish Male Smokers

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Background and Purpose—Homocysteine is associated with stroke, but it is not clear whether this relationship is causal. We examined the association between total serum homocysteine concentration (tHcy) and cerebral infarction in a cohort of Finnish male smokers.

Methods—This is a matched case-control study of 201 cases of cerebral infarction and 201 concurrently sampled age-matched controls nested in a cohort of 13 840 male smokers free of cardiovascular disease at the completion of the Alpha-Tocopherol and Beta-Carotene (ATBC) Cancer Prevention study. Conditional logistic regression was used to calculate odds ratios (ORs) and to adjust for confounding variables. An unmatched analysis was also performed.

Results—The geometric mean tHcy was 13.3 μmol/L (95% CI, 12.6 to 13.9) in cases and 12.6 μmol/L (95% CI, 12.0 to 13.2) in controls ($P=0.09$). There was a graded increase in the OR of cerebral infarction per quartile increase in tHcy (OR, 1.0, 1.7, 1.9, 2.1; trend $P=0.02$; 201 case-control pairs) when adjusted for traditional risk factors. There was a similar trend in a subgroup of 120 case-control pairs for which further adjustment for lifestyle factors was possible (OR, 1.0, 1.9, 2.5, 2.2; trend $P=0.07$ in the matched analyses; OR, 1.0, 1.2, 1.9, 2.0; trend $P=0.02$ in the unmatched analyses). The adjusted OR per 1-SD increase in log-transformed tHcy (equivalent to 4.7 μmol) was 1.4 (95% CI, 1.1 to 1.7; $P=0.01$).

Conclusions—tHcy appears to predict cerebral infarction in Finnish male smokers. **(*Stroke*. 2003;34:1359-1363.)**

Key Words: cerebral infarction ■ cohort studies ■ folic acid ■ homocyst(e)ine

Stroke, specifically cerebral infarction, remains an important cause of morbidity and mortality in developed countries. Moderately elevated total serum homocysteine concentration (tHcy) has been studied extensively as a potential independent risk factor for cerebral infarction and other cardiovascular diseases for several reasons. First, a human model of extreme disease exists; young adults with the severe genetic disease hyperhomocysteinuria and very high circulating levels of tHcy develop premature atherosclerosis and thromboembolism.[1] Second, several cellular mechanisms offer plausible pathogenic explanations.[2] Homocysteine causes oxidative damage to vascular endothelium with proliferation of vascular smooth muscle and creates a prothrombotic environment through its action on platelets, thrombin, and fibrin. Finally, tHcy can be lowered cheaply and easily with folic acid and other B vitamins.[3] Finding common preventable risk factors for cerebral infarction is desirable because of the potentially large public health impact.

The aim of this study is to examine the association between tHcy and cerebral infarction in Finnish male smokers. It contributes to the existing body of evidence in several ways. It has a larger number of cases than any previous nested case-control or cohort study. Unlike other studies, some of which include all clinical strokes, it includes only cases of cerebral infarction. Most important, to exclude the possibility of reverse causality, controls are selected in such a way as to exclude prevalent diagnosed and undiagnosed cardiovascular disease.

Materials and Methods

Alpha-Tocopherol, Beta-Carotene Cancer Prevention Trial

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study was a randomized controlled trial (RCT) set in Finland to examine the effects of alpha-tocopherol and beta carotene on cancer. In that study, 29 133 male smokers between 50 and 69 years of age were randomized between 1985 and 1988. The median duration of the trial was 6 years; it was terminated in 1993. Design, methods, patient characteristics, and compliance are described elsewhere.[4]

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Case Ascertainment

The study group continues to be notified, through record linkage, of incident events from the National Hospital Discharge Register and fatal outcomes via the Register of Causes of Death. This method of ascertainment was validated during the original trial. The diagnosis of cerebral infarction proved correct in 90% of hospital discharges and in 92% of death certificates in a sample of cases using standard diagnostic criteria.[5] When the trial ended in April 1993, 13 840 men were still alive and free of coronary heart disease and cerebral infarction. Cardiovascular disease end points ascertained previously were excluded. Subsequently, 574 of these men had a first coronary heart disease event or cerebral infarction between May 1993 and December 1995. One control, age matched within 4 years either side of the age of the index case (574), was selected concurrently by use of incident density sampling from all men still alive and free of cardiovascular disease at the time of the case event. Twenty-four men who were selected as controls became cases before the end of the sampling period. There were 212 cases of either incident or fatal cerebral infarction coded as ICD 433 to 434 (ninth revision of the *International Classification of Diseases*) during this time.

Measurement of Exposures

Participants in the ATBC study completed a baseline lifestyle questionnaire, including details on past medical history, occupation, smoking, alcohol consumption, and exercise. The Rose angina questionnaire and a dietary questionnaire were also completed. Physical examination included measurement of blood pressure, height, and weight.

Laboratory Methods

At baseline, serum samples were collected and stored at −70°C for future use. Frozen blood samples (n=1124) were transferred to the Institute of Clinical Science, Royal Victoria Hospital (Belfast, Northern Ireland) for analysis of total homocysteine, folate, serum cobalamin (vitamin B_{12}), and serum pyridoxal phosphate (vitamin B_6). Of 1124 unique blood samples, 1086 were suitable for analysis of tHcy, folate, and serum cobalamin, and 872 samples were suitable for analysis of serum pyridoxal phosphate. The laboratory staff were blinded to case-control status. Homocysteine, both free and protein bound, was assayed by high-performance liquid chromatography according to the method of Ubbink et al.[6] Concentrations of serum cobalamin and serum folate were measured by a competitive protein binding method with a SimulTRAC-S radioassay kit (ICN Pharmaceuticals). Pyridoxal-5-phosphate concentrations in serum were quantified with a high-performance liquid chromatography method by Reynolds and Brain.[7]

Statistical Analysis

Systolic and diastolic blood pressures, body mass index, total serum cholesterol, number of cigarettes regularly smoked per day, number of years of smoking, age at which the participant started smoking, and vitamin B_{12} were analyzed as continuous variables, and differences in means were estimated. Data on education attained were available as 6 categories ranging from less than primary school to high school graduate. Homocysteine, serum folate, and vitamin B_6 were not normally distributed but were log normal. Log-transformed data were used for comparing means at baseline, and geometric means are reported. Linear regression was used to examine the relationship between the natural log of Homocysteine and systolic and diastolic blood pressures. Crude and adjusted odds ratios of cerebral infarction were calculated by use of conditional logistic regression and estimated across quartiles of tHcy comparing each quartile to the first and tested for trend. As part of a sensitivity analysis and to increase statistical power, an unmatched analysis was done with a combination of both cerebral infarction controls and coronary heart disease controls. The odds ratio of cerebral infarction was calculated for a 1-SD increase in log-transformed homocysteine for the matched and unmatched groups. As they have been reported in other studies,[8–10] interactions between tHcy and cerebral infarction and blood pressure were examined through stratified analysis.

Results

There were 212 cases of cerebral infarction and 212 age-matched controls. Because of concurrent sampling, 9 controls later became cases and were included in the analysis twice. Of the matched pairs, 201 had complete tHcy measurements. Baseline characteristics for cases and controls and differences in means are shown in Table 1. The geometric mean tHcy was higher in cases than in controls, as were systolic and diastolic blood pressures, total serum cholesterol, daily consumption of alcohol, numbers of cigarettes smoked daily, and duration of smoking. Vitamin B_6 was slightly lower in cases than in controls, but serum folate and vitamin B_{12} were slightly higher in cases than in controls. Both systolic (β coefficient, 3.5; 95% CI, −0.3 to 7.2; $P=0.07$) and diastolic (β coefficient, 2.1; 95% CI, 0.08 to 4.2; $P=0.04$) blood pressures were associated with increasing homocysteine. Table 2 shows an increasing odds ratio of cerebral infarction across increasing quartiles of tHcy, comparing each quartile with the first and adjusting for systolic and diastolic blood pressures, total serum cholesterol, education, body mass index, duration of smoking, number of cigarettes smoked daily, age at which the participant started smoking, and trial treatment group. Further adjustment for serum folate, vitamin B_6, and alcohol in 120 case-control pairs for which there were complete data appears to strengthen the estimate. A fully adjusted unmatched analysis of the same 120 cases and 310 controls attenuates the effect slightly but showed a trend similar to the initial crude estimate. The odds ratios of cerebral infarction per 1-SD increase in logged homocysteine for the 3 different models are presented in Table 3. In men with and without elevated diastolic or systolic blood pressure, all odds ratios were similar (≈1.4) for a 1-SD change in tHcy.

Discussion

We have observed an increasing risk of cerebral infarction across increasing quartiles of tHcy in Finnish male smokers, with those in the top quartile of tHcy twice as likely to experience cerebral infarction as those in the bottom quartile. Overall, we have shown in 3 different samples that this corresponds to an increase in cerebral infarction events of between 20% and 40% for every 4.7-μmol/L increase in tHcy.

Several articles have demonstrated an association between elevated tHcy and stroke, but the point of this debate is whether they are causally related. The Vitamin Intervention for Stroke Prevention (VISP) trial,[11] an RCT of homocysteine-lowering B vitamins for the secondary prevention of nondisabling cerebral infarction, should demonstrate a secondary protective role if it exists. New evidence from an RCT of B vitamins after percutaneous angioplasty supports a secondary prevention role in cardiovascular disease,[12] although the same etiological mechanism may or may not apply in primary prevention. In the meantime, observational studies such as this provide the best available evidence of a primary effect. Cross-sectional[13,14] and case-control studies[15] are subject to selection bias and an interpretation of reverse causality, ie, that elevated homocysteine occurs as a result of cerebral infarction rather than causing it. Cohort studies or the more efficient nested case-control studies ensure that the

TABLE 1. Baseline Characteristics of Cerebral Infarction in Matched Cases and Controls

		Mean (SD)			
	n	Cases	Controls	Difference (95% CI)	*P*
Age, y	424	58.9 (5.2)	58.8 (5.3)	0.1 (1.1–0.9)	0.9
Blood pressure, mm Hg					
Systolic	424	147.7 (19.7)	143.6 (20.4)	2.1 (0.2–7.9)	0.04
Diastolic	424	90.1 (10.9)	87.8 (10.7)	2.2 (0.2–4.2)	0.03
BMI, kg/m^2	424	26.4 (3.5)	26.2 (3.8)	0.2 (0.5–0.9)	0.6
Cholesterol, mmol/L	422	6.4 (1.2)	6.2 (1.1)	0.2 (0.01–0.4)	0.07
Alcohol, g/d	380	20.1 (23.1)	19.1 (23.6)	1.0 (3.7–5.8)	0.07
Cigarettes smoked per day, n	424	20.1 (8.3)	19.9 (8.6)	0.2 (1.4–1.8)	0.8
Years smoked, n	434	37.4 (8.2)	36.1 (9.2)	1.3 (0.04–3.0)	0.1
Vitamin B_{12}, pmol/L	396	341.3 (100.5)	332.8 (111.9)	8.4 (12.6–29.4)	0.4
tHcys,* μmol/L	402	13.3 (5.2)	12.6 (4.8)	0.05 (−0.009–0.12)	0.09
Folate,* nmol/L	396	10.5 (4.7)	9.8 (4.5)	0.02 (−0.05–0.09)	0.6
Vitamin B_6,* nmol/L	270	26.6 (15.7)	28.1 (18.7)	−0.04 (−0.2–0.07)	0.4
Education achieved—primary school/lower, n (%)	424	162 (76.4)	162 (76.4)	0	
Intervention	424				
AT alone		49 (23.1)	60 (28.3)		
AT and BC		53 (25.0)	42 (19.8)		
BC alone		64 (30.2)	53 (25.0)		
Placebo		46 (21.7)	57 (26.9)	$\chi^2 = 4.6$	0.2

BMI indicates body mass index; AT, alpha-tocopherol; BC, beta-carotene.
*Geometric mean.

exposure is measured in an unbiased way before the outcome develops. Three cohort[9,16,17] and 5 nested case-control studies[8,10,18–20] have published findings on the association between tHcy and stroke in healthy population-based cohorts in whom homocysteine was measured with stored baseline blood and stroke, either all strokes or cerebral infarction only, was ascertained prospectively. All showed an increasing odds ratio of stroke with increasing tHcy, but several showed small, weak effects and were underpowered. Several systematic reviews and meta-analyses have also been published[21–26]; the most informative is an individual patient data reanalysis.[27] That study demonstrated an adjusted odds ratio of stroke in the prospective studies (463 strokes) of 0.83 (0.77 to 0.89) per 25% lowering of usual tHcy concentration (expected with folic acid supplementation), equivalent to about 3 μmol/L tHcy. Our estimate of effect is consistent with these results.

A number of design features that have not been incorporated into previous studies contribute to the validity of this study. It has the largest number of cases in any single study to date, resulting in more statistical power to detect a small effect. Some studies of tHcy and stroke included all strokes[8,17,19] without separating stroke subtypes with different pathogeneses. This could potentially dilute the estimate of effect toward the null. We therefore included only cases of cerebral infarction that had been ascertained with validated methods. We made extensive effort to minimize reverse causality by appropriate selection of cases. Prevalent undiagnosed cardiovascular disease (both cerebral infarction and

TABLE 2. Crude and Adjusted Odds Ratios (95% CIs) of Cerebral Infarction Comparing Each Quartile of the tHcy Distribution to the First

Quartile	tHcy, μmol/L	Crude, 201 Pairs	Model A, 201 Pairs	Model B, 120 Pairs	Model C, 120 Cases, 310 Controls
1	3.1–10.5	1.0	1.0	1.0	1.0
2	10.6–12.6	1.5 (0.8–2.6)	1.7 (0.9–3.1)	1.9 (0.6–5.5)	1.2 (0.6–2.4)
3	12.7–15.4	1.7 (1.0–3.0)	1.9 (1.1–3.2)	2.5 (1.1–6.6)	1.9 (1.0–3.6)
4	15.4–86.2	1.9 (1.0–3.4)	2.1 (1.1–3.9)	2.2 (0.8–6.3)	2.0 (1.0–4.0)
	P trend	0.02	0.02	0.07	0.02

Model A: Adjusted for systolic blood pressure, diastolic blood pressure, total serum cholesterol, education, body mass index, duration of smoking, number of cigarettes smoked daily, age started smoking, and trial treatment. Model B: model A plus serum folate, serum vitamin B_6, and alcohol. Model C: model A plus model B plus age (unmatched analysis).

TABLE 3. Adjusted Odds Ratios of Cerebral Infarction per 1-SD* Increase in tHcy

		n	Odds Ratio	95% CI	P
Crude	201 matched case-control pairs	402	1.2	1.0–1.4	0.1
Model A	201 matched case-control pairs	402	1.2	0.9–1.4	0.1
Model B	120 matched case-control pairs	240	1.3	0.9–1.7	0.1
Model C	120 cases, 310 controls, unmatched	430	1.4	1.1–1.7	0.01

See Table 2 for description of models.

*Corresponds to 4.7 μmol/L Hcy on the normal scale.

coronary heart disease) is excluded by only ascertaining cases after the trial was terminated; ie, all incident cases occurring within the first 6 years (median) were excluded. Prevalent diagnosed coronary heart disease and cerebral infarctions were also excluded because case status was determined at the first cardiovascular disease end point.

We aimed to select the most appropriate control group possible by using concurrent sampling. Also known as incident density sampling or risk-set sampling,[28] in this method, controls are randomly selected from those at risk of the disease at the time of the event as opposed to being selected from survivors at the end of the follow-up period. Controls are eligible to become cases later and are included in the analysis twice. In this study, 9 controls later became cases of cerebral infarction. Concurrent sampling confers 2 major advantages. The first is that the odds ratio is closer to the true rate ratio of disease because the probability of being selected as a control is proportional to the number of person-years-at-risk contributed by the individual. The second is that selection bias of healthy survivors is avoided.

A previous Finnish cohort[19] from Kuopio demonstrated almost no effect of tHcy on stroke or coronary heart disease. The mean and range of tHcy in that study were low relative to other populations (9.8±3.2 μmol/L). One explanation was the low frequency of genes that predispose to hyperhomocysteinemia (MTHFR C677T) in the Finnish population, and in combination with a small number of cases, this cohort study did not have enough statistical power to detect an effect. Our study had a higher geometric mean tHcy (12.6±4.7 μmol/L) than the Kuopio study. This could be due to age or laboratory methods, but a more likely explanation is that all the ATBC participants were smokers. The Hordaland Homocysteine study[29] showed a strong graded increase in tHcy with number of cigarettes smoked. The adjusted estimate of the difference in tHcy between smokers and nonsmokers was ≈1.9 μmol/L. In this population of Finnish smokers, the tHcy distribution is shifted to the right by ≈2 μmol/L, relative to the range in the Kuopio study. The results could therefore mean 1 of 2 things. First, the effect of tHcy on cerebral infarction is greater within a higher range of tHcy, and the cause of the higher range is not important. Alternatively, there may be an interactive effect with smoking, and the association within smokers is different from that in nonsmokers.

Many but not all studies describe a strong positive association between smoking and tHcy and adjust for its confounding effect. Only 1 study, a large case-control study, has described an interaction with smoking in which the observed odds ratio of all cardiovascular disease in smokers with elevated tHcy and hypercholesterolemia was 4.6 compared with an expected additive estimate of 3.3.[15] In the Caerphilly cohort,[9] there was an interaction in the opposite direction, although the numbers were small. Nonsmokers had a higher hazard ratio of stroke per 1-SD change in log-transformed tHcy (1.3; 95% CI, 1.0 to 1.7; 51 cases, 1272 controls) compared with current smokers (1.0; 95% CI, 0.8 to 1.3; 56 cases, 976 controls). It may well be that the strength of effect is the same in smokers and nonsmokers and that this study is demonstrating the true estimate of effect in the normal population. The mean and distribution of tHcy in the ATBC study are similar to those of the British Regional Heart Study,[8] less than those in the Rotterdam study,[10] and slightly more than those in Caerphilly.[9] Different laboratories could account for these small differences, but smoking itself does not appear to elevate homocysteine noticeably compared with these other cohorts.

Residual confounding must be considered as an explanation for our results. We have been able to adjust for many of the major lifestyle factors and dietary factors associated with hyperhomocysteinemia.[30] This is a cohort of smokers, but we adjusted our estimate of effect for all variations of smoking, including age started, number of cigarettes smoked, and duration. Hypertension, not strongly associated with tHcy in this cohort but an important confounder in other studies, was also taken into account. Further analysis was made for serum B vitamins, which reflect dietary intake, are closely associated with tHcy, but could have an independent effect on cardiovascular disease outcomes. We also adjusted for body mass index. Unfortunately, serum creatinine or other measures of renal impairment were not available. Renal impairment at baseline could explain this result, but the exclusion of incident events during the trial over a median of 6 years would reduce this effect. The most striking thing about these results is that adjusting for different confounders changes the crude estimate of effect very little, and overall, the amount of confounding is small.

Many of the biochemical samples were inadequate for biochemical analysis data, particularly vitamin B_6. These losses were amplified because of the methodology used. In a matched analysis, if data are missing for 1 subject, the pair is not included. In concurrent sampling, missing data for 1 subject who is both case and control for 2 different pairs result in the exclusion of both pairs. This is unlikely to explain our results because loss of samples was equal in both cases and controls.

Previously, we reported an adjusted hazard ratio of ischemic stroke of 1.3 (95% CI, 1.1 to 1.7) in hypertensive men versus 0.8 (95% CI, 0.6 to 1.2) in normotensive men in the Caerphilly cohort.[9] Other studies have reported a similar interaction. There was no robust evidence of an interaction between the effect of tHcy on stroke and hypertension in the ATBC cohort. We were unable to look for a differential effect of age because of matching.

This study addresses some of the shortcomings of previous prospective studies. It is a well-conducted study with a large sample size, valid case ascertainment, appropriate sampling of controls, exclusion of prevalent disease, and adjustment for most of the major confounders. It shows a consistent graded increase in risk of cerebral infarction with increasing tHcy. The overall effect is small, between 20% and 40% per 4.7-μmol/L increase in tHcy, and may or may not be exclusive to smokers. However, cerebral infarction is common, and elevated tHcy is treatable. Even a small reduction in risk could have a substantial public health impact.

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